January 30, 2004

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Re: Comments of the Chlorine Chemistry Council on the Draft NTP Technical Reports on the Toxicology and Carcinogenesis Studies of 2,3,4,7,8-PeCDF (NTP-TR-525), 2,3,7,8-TCDD (NTP-TR-521), and a Mixture of 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF, and PCB 126 (NTP-TR-526).

The Chlorine Chemistry Council® (CCC) appreciates this opportunity to comment on the National Toxicology Program's (NTP) draft reports. CCC, a business council of the American Chemistry Council, is dedicated to addressing public policy issues affecting chlorine chemistry, including efforts to study the toxicology and carcinogenesis of dioxin and dioxin-like compounds. Because NTP's studies have the potential to impact the use of the toxic equivalency factor (TEF) methodology and its adequacy for predicting relative potency for cancer risk, CCC has a strong interest in the studies and their interpretation. CCC believes there are both valuable precepts, as well as a number of problems, inherent with the draft reports.

CCC's comments include both general observations on the three studies and specific comments on the individual reports.

General Observations

NTP should devote a greater discussion to the pathology of the lesions found throughout the studies. Specifically, more discussion is warranted concerning the difficulties encountered with diagnosing and confirming hyperplastic nodules versus adenomas versus carcinomas of the liver. Similarly, more discussion should be devoted to the findings and apparent reversal of pre-neoplastic and neoplastic lesions in the start-stop study. Perhaps a brief overview of the classification scheme used for the most difficult proliferative lesions to categorize would be appropriate. Additionally, mention of and reference to the *Special Pathology Workshop on Hepatocellular Proliferative Lesions Associated with Dioxin and Dioxin-Like Compounds* Report issued November 12, 2003 might also be useful. In fact, it would be appropriate to cite this document on a number of occasions throughout each report.

In the discussion of the toxicity of dioxin and dioxin-like compounds, NTP makes some overly broad statements that might be misconstrued or otherwise taken out of context. For instance, statements such as "high doses/or continuous exposure to dioxin leads to a broad spectrum of toxic responses including … impaired reproduction and development" or "toxic effects include … carcinogenicity and lethality" are potentially misleading statements because they do not clearly articulate that these responses are based on observations from experimental animals. Without this important clarification, the statement implies that these effects have been reported in highly exposed human populations, which is not true. In general, any discussion of known

toxicological observations for TCDD and dioxin-like compounds should include the scientific basis upon which these observations are based.

Specific Comments

NTP Technical Report on the Toxicology and Carcinogenesis of 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) (CAS No. 57117-31-4) in Female Harlan Sprague-Dawley Rats (Gavage Studies) (NTP-TR-525)

- NTP's discussion of human exposure to PeCDFs (p. 33) should be more balanced. For instance, NTP might include a brief mention of the declining environmental trends and body burdens of TCDD and dioxin-like compounds. Such a discussion could include a number of recent studies, such as Lorber (2002), Hays and Aylward (2003), and Ewers et al. (1996).

- Likewise, NTP's section on toxicokinetics (pp. 33-34) should mention the recent PBPK modeling work by Aylward et al. (2003a). This work should be included in each discussion of toxicokinetics and dioxins.

- The section concerning carcinogenicity in humans (p. 36) mentions "extensive reproductive and developmental effects" in the Yusho and Yucheng populations as a result of PCDF exposure, yet "extensive" is not defined. The specific dermal and developmental effects should be clarified so that the reader is not left with the misunderstanding that any and all types of reproductive and developmental effects have been reported in overt poisoning instances where humans have accidentally ingested μg/kg dosages of PCBs, chlorinated furans, and quarterphenyls over a short period of time.

- This carcinogenicity section also cites the IARC 1997 document, yet fails to make clear to the reader that IARC concluded the epidemiological data was *limited*. It was only when IARC combined this *limited* data with mechanistic data that IARC classified TCDD as carcinogenic to humans.

- In the section of the report, entitled "Tumor Promotion Studies" (p. 37), Waern et al. (1991) is cited with reference to a TEF five-times less potent than TCDD. However, this is an incomplete presentation of that study. Based on liver concentrations, a TEF of 0.007 was estimated by Waern et al, an estimate that is approximately 143 times less potent than TCDD.

- The "Mechanism and Biochemical Effects" section (p. 38), contains the statement, "In general, the potency of effects of PCDD and PCDFs exhibit a rank order potency similar to that seen for relative binding to AhR." However, this conclusion has never been supported by a rigorous statistical review of the data. A closer inspection of the dose response data demonstrates the same lack of dose-response additivity (different slope and different maximal response) that Toyoshiba et al. (2004) found for the new NTP enzyme induction data. More discussion should be included of the uncertainty that exists in TEFs

published in Van den Berg et al. (1998). Furthermore, a similar discussion of the Toyoshiba et al. (2004) study on the enzyme induction results would be appropriate and useful for the "Discussion and Conclusion" section of the report.

- Page 104 contains the statement "The heart is a known target for TCDD and related dioxin-like compounds in both rodents and humans." This statement is inaccurate because the epidemiological evidence linking TCDD and dioxin-like compounds to heart disease in humans is not conclusive. For example, the basis of the Flesch-Janys (1995) associations rests on a first-order pharmacokinetic clearance of dioxin in the high exposure group for which a weak association was reported for heart disease. However, high dosages incurred by workers results in a second-order clearance rate leading to a much higher estimate of body burdens for highly exposed workers (Aylward et al. 2003b). Therefore, the weak associations of Flesch-Janys et al. (1995) require a careful re-evaluation, in light of these new PBPK data, to see if they remain supportable. Additionally, other studies, such as the much larger NIOSH cohort (Steenland et al., 1999) do not support a causal association between TCDD exposure and heart disease mortality. This observation is also applicable for the NTP report on TCDD (NTP-TR-521).

- Finally, the PeCDF report throughout compares rare tumor occurrences in the treated groups to the combined control groups from the four NTP dioxin carcinogenicity studies. This is not scientifically supportable since the four studies do not provide sufficient historical context for this type of comparison. A mere four studies with approximately 200 control rats should not be characterized as a historical control population. Historical controls generally reflect a long-term experience with the species and strain, capable of demonstrating genetic drift and a good deal of robust statistical value. This does not exist, however, for female Sprague-Dawley rats at NTP. More caution would be preferable when comparing the rare cancer observations in treated groups to the very limited historical control group that exists within NTP.

NTP Technical Report on the Toxicology and Carcinogenesis of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (CAS No. 1746-01-6) in Female Harlan Sprague-Dawley Rats (Gavage Studies) (NTP-TR-521)

- Page 10 of the "Executive Summary" contains the statement, "At 2 years, the incidence of squamous cell carcinoma of the uterus in the 46 ng/kg group was significantly increased, and there were two squamous cell carcinomas in the 100 ng/kg stop-exposure group." However, since there was no dose-response evidence (i.e., no uterine tumors at 100 ng/kg/day), the apparent increase was of questionable treatment-related nature and should be reflected within the text.

- Also on Page 10, mention is made of a historical control database. As previously stated, any references to this historical control group should be tempered with some measure of caution.

- A sentence on page 69 referring to Table 11 reads, "The labeling index was significantly higher in all dose groups at the 31-week interim than in the vehicle controls." However, this appears as an artifact of a control value that is too low (i.e., 0.327 % labeling index versus 1.410 and 0.948 in the other two control values). Since these are mature rats, the control labeling percentage across controls should be relatively constant. Hence, a more appropriate technical evaluation of the 31-week data should consider the abnormally low control value, as well as the control data for the other two time points (14 and 53 weeks).

- The "Discussion and Conclusion" section on page 101 contains the statement, "A quantitative analysis of the effects observed in this study to responses observed with other compounds studied as part of the Dioxin TEF Evaluation will be presented in a future Technical Report." We recommend that the Toyoshiba et al. (2004) paper, along with its purpose and conclusions, be considered for inclusion in this analysis, especially since it has been published in a peer-reviewed journal. It is recommended that Toyoshiba et al. (2004) be cited, as well as discussed extensively.

NTP Technical Report on the Toxicology and Carcinogenesis of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) (CAS No. 1746-01-6), 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) (CAS No. 57117-31-4), and 3,3',4,4',5-Pentachlorobiphenyl (PCB 126) (CAS No. 57465-28-8) in Female Harlan Sprague-Dawley Rats (Gavage Studies) (NTP-TR-526)

- Page 35 contains the statement, "…it has been estimated that the whole body half-life of TCDD in humans is approximately 7 to 10 years." This statement is inaccurate, however, particularly in light of the recent work published by Aylward et al. (2003b). Using the Carrier and Brunet PBPK model, Aylward et al. (2003b) demonstrated that clearance in humans is a second order phenomenon related to CYP1A2 levels that are dose-dependent. Much shorter half-lives occur for highly exposed populations such as Seveso and workers. The 7-10 year half-life estimates, on the other hand, are only valid for the terminal elimination phase where liver concentrations are in the low 100 ppt concentrations or lower. Also within this section, the Aylward et al. (2003b) study should be cited along with USEPA (2000b) (p. 35) indicating dose-dependent clearance for TCDD.

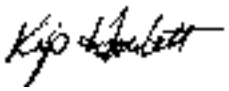
- Page 38 contains a discussion of epidemiological data that is inaccurate and perhaps beyond the scope of this report. For example, as previously discussed, IARC (1997) concluded that the epidemiological evidence for TCDD's human carcinogenicity was *limited*. We suggest that the report refer the reader to IARC, or more recent reviews on the epidemiological evidence, such as Crump et al. (2003), Starr et al. (2003), and Cole et al. (2003).

- Finally, a more extensive discussion on the failure of the applied dose to create target organ concentrations consistent with the TEF estimates would be useful in this report. This might serve as a proper starting point to further discuss TEF estimate derivations based on the administered dose versus TEF estimates based on target organ concentrations.

Overall, CCC believes that each draft report has merit and is technically sound. However, we recommend that NTP devote greater discussion to the pathology of the lesions found throughout the studies, and that discussions of toxicological observations for TCDD and dioxin-like compounds clearly articulate the scientific basis for these observations, so that the reader does not inappropriately assume that observations based on animal studies necessarily apply to humans. More specific comments for each report are noted above.

Please direct any questions or comments you may have concerning this submission to Todd Abel (703-741-5856).

Sincerely,



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Vice President

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